Biochemistry

Part 2

Protein Dynamics

Protein Dynamics (Part two)

Objectives

- Describe the thermodynamics of reactions including when a reaction is spontaneous or non-spontaneous.
- Describe the function of enzymes and their effect on reaction thermodynamics.
- Define the different reactions that commonly named enzymes complete.
- Define the different types of inhibitors and their effect on enzymatic reactions.
- Draw the plot of the velocity of an enzymatic reaction versus the substrate concentration (Michaelis-Menten plot), with and without inhibitors, and label Vmax and Km.
- Draw and label the Lineweaver-Burk plot.

In Part 2, protein dynamics are discussed including thermodynamics, enzyme kinetics and specificity, and inhibition of enzymes.

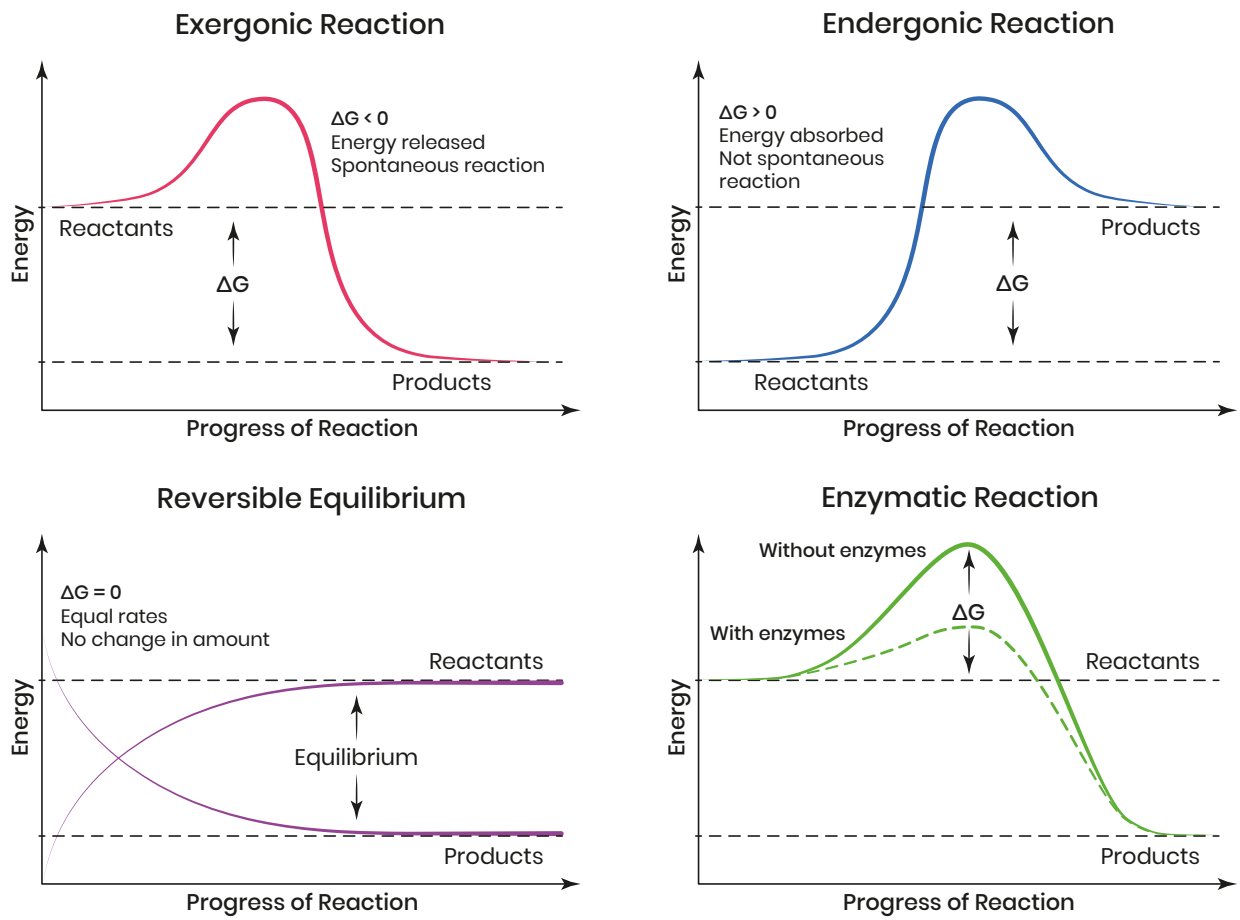
Thermodynamics is a branch of physics that includes heat and its relation to other forms of energy. Reaction thermodynamics involves the heat and energy of chemical reactions. Chemical reactions start with reactants, a reaction takes place, and products are made. Both the reactants and the products have energy, mostly potential energy, just based on their location, charge, and composition. When reactions take place, the starting energy of the reactants and the energy of the products can be measured to determine whether a reaction will require energy (**endergonic** reaction), will produce energy (**exergonic** reaction), or will not produce or require energy (a reaction at equilibrium). Reactions also require **activation energy**, energy that must be added to the system for the reaction to occur. Once the energy of the reactants, activation energy, and energy of the products is known, it is possible to determine if a reaction is exergonic or endergonic (Figure 37). Nearly all chemical reactions in cells are carried out by **enzymes**, proteins (and sometimes RNA) that are specialized catalysts that increase the rate of chemical reactions by lowering the activation energy (Figure 37).

Enzymes have specificity because they have an active site where only a certain reactant (substrate) fits, similar to a lock and key. There are several important facts regarding enzymes:

- Enzymes themselves do not change during a reaction, they are not used up in the reaction and therefore are able to continue to catalyze a reaction as long as they have reactants
- Enzymes do not change the overall energy of a reaction because they do not affect the starting energy of the reactants or the ending energy of the products, they simply reduce the activation energy so that products are reached more quickly
- Enzymes cannot make an endergonic reaction exergonic; again, enzymes do not change the energy of the reactants and products
- Enzymes have a maximum speed that they can catalyze a reaction, often dependent on the concentration of reactants, temperature, pH, affinity of the enzyme to the reactants, and presence of inhibitors. Temperature and pH are often assumed to be constant when considering reactions occurring in the body.

Thermodynamics of reactions

Figure 37



The thermodynamics of reactions that are spontaneous, non-spontaneous, at equilibrium, and those catalyzed by enzymes plotted as a function of change in free energy (delta G) from reactants to products versus the progress of the reaction. The plot on the bottom right shows the effect of an enzyme catalyzing a reaction (dotted line); enzymes lower the activation energy, but do not change the overall energy of the reactants or products.

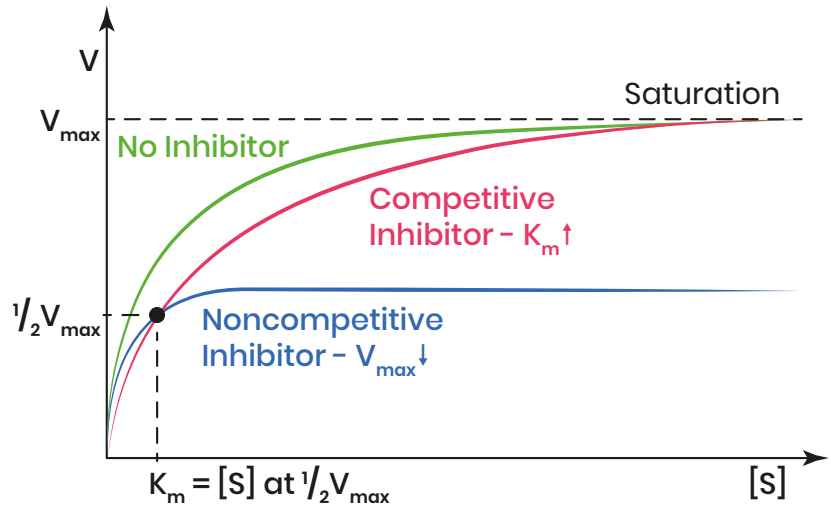
As noted above, enzymes are specific for the reaction they catalyze, so there are hundreds of thousands of enzymes, but there are some naming conventions that help describe the type of reaction an enzyme catalyzes. Some common names are listed below:

Enzyme Name	Reaction Catalyzed
Carboxylase	Transfer of CO_2 groups. Requires the coenzyme biotin (B_7)
Dehydrogenase	Oxidation-reduction reactions
Hydroxylase	Addition of hydroxyl group
Kinase	Transfer of a phosphate group from a high energy molecule to a substrate (usually from ATP to a substrate)
Mutase	Movement of functional groups within a molecule
Phosphatase	Removes a phosphate group from a substrate
Phosphorylase	Adds a phosphate group from an inorganic source (not ATP) to a substrate
Synthase	Combines two molecules

Enzyme kinetics is the study of the chemical reactions catalyzed by enzymes and the conditions that affect enzymatic reactions. As noted above, each enzyme has a maximum speed at which it can catalyze a reaction (called velocity max or Vmax), which is dependent on the concentration of substrate, temperature, affinity of the enzyme for the substrate, and presence of inhibitors. Reactions catalyzed by enzymes can be graphed by plotting the velocity of the reaction against the concentration of substrate as show in Figure 38. The plot is a graphical representation of the **Michaelis-Menten equation:** velocity=(Vmax [S])/(Km+[S]), where [S] is the concentration of substrate. Km is a constant that is inversely related to the affinity of the enzyme for the substrate (higher affinity=lower Km). Km is equal to [S] at ½ Vmax. As the graph shows, reaction velocity increases with increasing substrate concentration to a point, Vmax, after which the enzyme is said to be saturated, and cannot react any faster. Note: Most enzymes have kinetics that follow hyperbolic curves as shown in Figure 38, however, there are exceptions. For example, hemoglobin exhibits cooperative binding, and has a sigmoidal reaction curve.

Michaelis-Menten model of enzyme kinetics

Figure 38



The enzyme reaction is shown without inhibitor, with a competitive inhibitor, and with a non-competitive inhibitor; note the effect of different types of inhibitors on the Vmax as well as the Km.

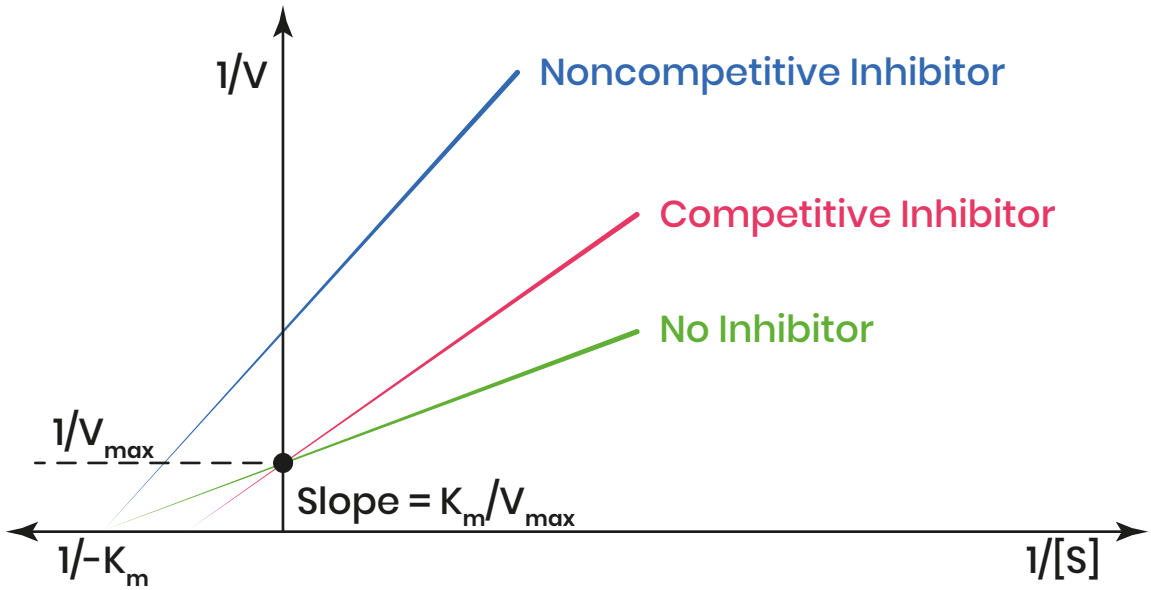
Inhibitors are molecules that bind to enzymes and decrease the enzyme's activity. There are two main types of inhibitors: competitive and non-competitive (Figure 38).

- **Competitive Inhibitor:** Binds to the enzyme in the active site and blocks the enzyme from binding the substrate. Competitive inhibitors can be reversible or irreversible, if they are reversible, they can be overcome by adding more substrate. Higher Km, no effect on Vmax. Reversible competitive inhibitors decrease potency, irreversible competitive inhibitors decrease efficacy.
- **Non-competitive Inhibitor:** Binds to the enzyme at a site other than the active site, but alters the structure of the enzyme so that the enzyme functions less efficiently; as a result, cannot be overcome by increasing substrate concentration. Lower Vmax, no effect on Km. Noncompetitive inhibitors decrease efficacy.

Another way that enzyme kinetics are frequently displayed is by using a Lineweaver-Burk plot, which plots 1/velocity versus 1/[S], thereby making enzymes with hyperbolic curves have linear binding curves (Figure 39). It is important to note that 1/-Km is the x-intercept of the plot, slope=Km/Vmax, and the y-intercept is equal to 1/Vmax. Also shown in Figure 39 are the plots of competitive and non-competitive inhibitors (note how competitive inhibition results in a higher Km, lower affinity).

Lineweaver-Burk model of enzyme kinetics

Figure 39



The reaction is shown without an inhibitor, with a competitive inhibitor, and with a noncompetitive inhibitor. Note how each different inhibitor affects the Km and Vmax.